AD-A283 884

1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED

4. TITLE AND SUBTITLE

Neurotoxicity in Animals Due to Arteether and Artemether

5. FUNDING NUMBERS

WR-093-94

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Walter Reed Army Institute of Research
Washington, DC 20307-5100

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research, Development, Acquisition and Logistics Command
Ft. Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

APPROVED FOR PUBLIC RELEASE:
DISTRIBUTION UNLIMITED

12b. DISTRIBUTION CODE

F

13. ABSTRACT (Maximum 200 words)

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14. SUBJECT TERMS

Artemisinin, Qinghaosu, Arteether, Artemether

15. NUMBER OF PAGES

4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)

Neurotoxicity in animals due to arteether and artemether

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Abstract

Several artemisinin (qinghaosu) derivatives have been developed and are in use as antimalarial drugs but scant animal or human toxicity data are available. We noted a progressive syndrome of clinical neurological defects with cardio-respiratory collapse and death in 5/6 dogs dosed daily for 8 d with intramuscular arteether (AE) at 20 mg/kg/d in a pharmacokinetic study. Neurological findings included gait disturbances, loss of spinal reflexes, pain response reflexes and prominent loss of brain-stem and eye reflexes. Electrocardiography showed prolongation of the QT interval corrected for rate (QTc). Prominent neuropathic lesions were sharply limited to the pons and medulla. Neurological injury, graded by a pathologist 'blinded' to dose group, showed a dose-related region-specific injury which was most pronounced in the pons and medulla in all animals. Rats treated with AE and artemether (AM) at 12·5 to 50 mg/kg/d for 28 d confirmed clinical neurological abnormalities with high doses (>25 mg/kg/d) after 6–14 d. Neuropathological examination of rat brain sections at 5 levels from the rostral cerebrum to the caudal medulla showed a dose-related pattern of injury characterized by hyalinized neuron cell bodies and loss of Nissl substance; changes congruent with those noted in dogs. No significant difference was noted in the extent, type, or distribution of lesions in the brains of rats treated with equivalent doses of AE or AM. We conclude that (i) a neurological syndrome with central nervous system neuropathological changes occurred in a dose-related, and anatomically specific manner in both dogs and rats given moderately high daily doses of AE or AM; (ii) prolonged QTc interval was a preterminal clinical finding in dogs and rats treated with high dose AE; (iii) the mechanism and aetiology of these lesions was not determined in this study but a long-lived toxic drug metabolite is suggested.

Introduction

The discovery of qinghaosu (artemisinin) by the Chinese and identification of its unique sesquiterpene lactone endoperoxide structure was an important milestone in antimalarial chemotherapy. Antimalarial activity is increased by reducing the lactone oxygen to yield dihydroartemisinin which can be further derivatized to ether or ester analogues (KLAYMAN, 1985). Two derivatives of artemisinin include methylether (artemether) and ethylether (arteether) forms, which appear to have similar physical/chemical and antiparasitic properties (SHMUKLARSKY *et al.*, 1993). Artemisinin and arteether have been widely reported by the Chinese and others to have been successfully used as a blood schizontocide, formulated as an intramuscular (i.m.) peanut oil solution or oral capsules (HIEN & WHITE, 1993). Very little clinical toxicity other than occasional changes in the electrocardiogram has been noted in clinical use and in the few published animal toxicology studies (HIEN & WHITE, 1993).

The rapid onset of action, activity against drug resistant malaria, and low reported toxicity have led to considerable interest in this group of drugs. Arteether was selected by the US Army and the World Health Organization (WHO) for development as an i.m. sesame oil solution for emergency treatment of severe malaria—especially cerebral malaria.

Early animal toxicology studies showed occasional unexplained deaths in animals receiving high doses of the compounds. Abnormalities in the electrocardiogram (ECG) with prolongation of the QT interval had been noted in animal and human studies, so the unexplained deaths were presumed to be cardiovascular. During the course of multiple-dose pharmacokinetic studies in our laboratory, sudden unexplained neurological findings and death were noted in several animals given high doses of i.m. arteether. These observations led to the investigations described below. Our studies demonstrated a delayed onset, dose-dependent central nervous system toxicity with a unique region-specific distribution in rats and dogs after repeated dosing with either arteether or artemether. This report describes the nature and extent of these neuropathic lesions and associated observations.

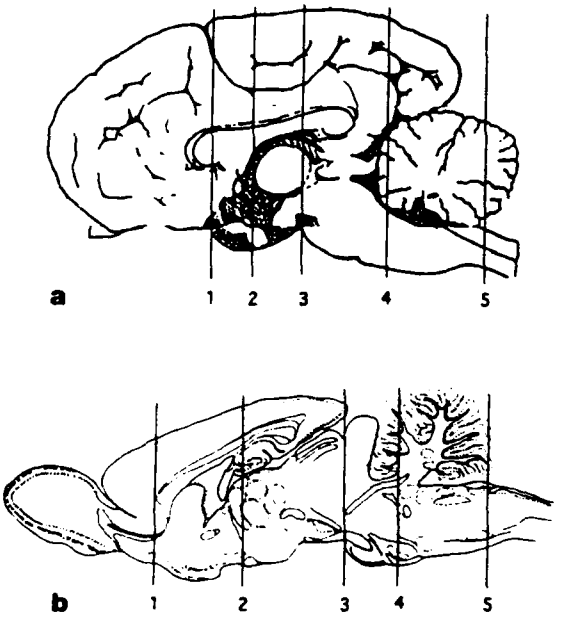

Fig. 1. (a) Schematic sagittal outline of dog brain with section levels indicated by numbered vertical lines. Level 1, immediately anterior to union of optic nerve; level 2, through the centre of the tuber cinereum; level 3, immediately anterior to the pontal protuberance; level 4, through the posterior pontal protuberance at the base of the trigeminal nerve; and level 5, distal to the hypoglossal nerve. (b) Outline of sagittal rat brain showing section levels by numbered lines. (Brain figures modifed from PELLEGRINO *et al.*, 1979; LIM *et al.*, 1960.

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All animal studies were conducted in compliance with the NIH Publication No. 86-23, 'Guide for the Care and Use of Laboratory Animals', revised 1989; Animal Welfare Act of 1966, as amended in 1970, 1976, and 1985; and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, revised 1986.

The views expressed herein are those of the authors and do not necessarily represent those of the US Department of Defense or the United States Army.

Materials and Methods

Drugs used were prepared under contract to the US Army, analysed for purity, and formulated in US Pharmacopeia (USP) sesame oil under sterile conditions.

Two multiple dose studies were completed in dogs; one in which arteether in sesame oil was administered daily (i.m.) for 8 d at 10 or 20 mg/kg/body weight/d ($n=6$ per group) and a subsequent confirmatory study in which dogs were dosed with i.m. arteether at 5, 10, 15 and 20 mg/kg/d for 28 d ($n=2$ per group). Perfusion-fixed brains were sectioned at 5 levels (Fig. 1, a) and coded slides were examined by a pathologist (S.J.G.) 'blinded' to treatment and scored according to severity of neuropathic lesions from 0 (unaffected) to 4 (multiple areas within each section with numerous degenerative nerve cells).

Studies with rats were carried out using adult males housed in individual cages and dosed i.m. with vehicle control (sterile USP sesame oil), arteether or artemether at 12·5, 25 or 50 mg/kg/body weight ($n=6$ per group). Perfusion-fixed brains and spinal cords were serially sectioned at 5 levels of the central nervous system corresponding to the levels evaluated in the previous dog studies (Fig. 1, b). Slides were examined 'blind' and scored (by S.J.G.) on a 0 to 5+ severity scale similar to that used for the dogs. Telemetry ECGs from rats dosed with arteether (50 mg/kg/body weight) were evaluated for rate, mechanism, arrhythmias, ectopy and intervals, under software control and from printed recordings.

Statistical analysis

Animal weights and telemetry ECG data were analysed as continuous data, while brain section level and neuropathic scores were treated as nominal and ordinal data, respectively. Data from rat ECGs were compared using the paired t test. Differences between drug treatment groups were assessed using Mann-Whitney U (2 groups) or Kruskal Wallis tests (≥ 3 groups) (SNEDECOR & COCHRAN, 1968). Relationships between drug dose and neuropathic severity scores were evaluated using logistic regression analysis (HOSMER & LEMESHOW, 1989). Analyses were performed using Minitab (MINITAB, 1990) and SAS JMP (SAS, 1989) on VAX 1170 and Apple MacIntosh II computers respectively.

Results

Clinical neurological findings in multiple-dosed dogs

Plasma levels of arteether were determined by high performance liquid chromatography using reductive electrochemical detection (MELENDEZ *et al.*, 1991) and showed a dose related increase in plasma level until day 5 (data not shown). No neurological finding was noted in low dose groups (5 and 10 mg/kg) in either study, but animals in high dose groups demonstrated depressed sensorium with abnormal neurological findings (see below) and progressive ataxia, with death of 6 of the 10 high dose animals in both studies. ECGs of animals with neurological findings exhibited a prolonged QTc interval (QT interval corrected for rate) (GALLAGHER, 1992; TODT *et al.*, 1992) without arrhythmias or ectopy.

Both high-dosed and low-dosed animals were examined neurologically. No significant, reproducible neurological deficit was noted in low-dosed animals, while all high-dosed dogs showed neurological deficits, albeit to different degrees. In low-dosed animals, all tested reflexes, including cranial nerve function by menace reflex, vestibular nystagmus (dolls' eyes), and pupillary light reflex were normal. Spinal reflexes (patellar, tibial, biceps, triceps, perineal, panniculus and deep pain reflexes) were intact. Postural reactions including 'wheel-barrowing', hopping, extensor postural thrust, hemi-stand, hemi-walk, proprioceptive and optic/tactile placing, were also intact. The most severely affected high-dosed animals were unable to stand or walk and, despite their having eyes open, did not spontaneously track objects or have a menace reflex or response to visual/auditory environmental stimuli. Normal vestibular nystagmus was absent but the pupillary light reflexes were normal bilaterally. All spinal reflexes were present and deep pain reflexes were normal. All postural reflexes examined were absent. Animals demonstrated rapid shallow respiration, which became progressively depressed until respiratory arrest supervened.

Clinical and neurological changes in multiple dosed rats

Clinical findings in rats comprised dose-related changes in growth and weight gain and mortality rate between the controls and the animals receiving the 3 dose levels of arteether or artemether. A number of animals had neurological changes, which included abrupt onset of ataxia as well as spontaneous myoclonic-like activity, in the 1–2 d before death.

Electrocardiographic changes

Serial ECG recordings from rats showed marked changes in the ST–T wave morphology, inversion of the

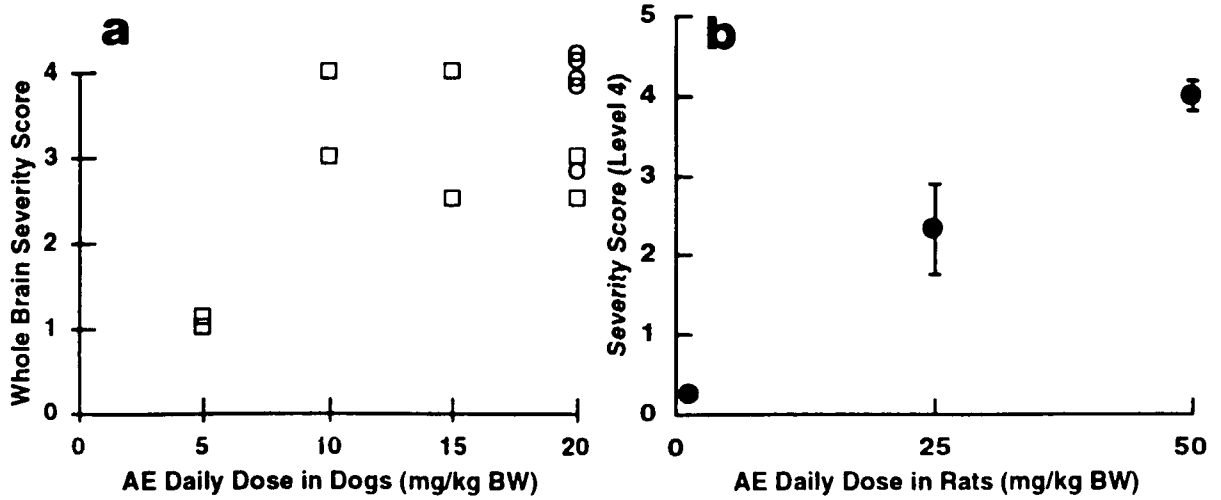

Fig. 2. Lesion severity versus dose of arteether (AE). a: Plot of lesion severity scores assessed for the whole brain of each dog evaluated in two separate studies (○, 20 mg kg d × 8; □, 5–20 mg kg d × 28). There is a significant ($P<0.01$) relationship between daily dose of arteether and severity by both linear regression and the more appropriate logistic regression analysis. b: Severity score (mean + SD) for rats treated with arteether (0–50 mg kg d × 14, $n=6$ per group). The score is highly dose dependent ($P<0.01$, logistic regression analysis for ordinal data) in all caudal sections; data for level 4 are shown. (BW = body weight)

T wave and increased QTc duration ($P<0.05$) 72–96 h before the animal exhibited clinical abnormalities (data not shown).

Neuropathological description

Dogs. Brain lesions in all dogs from both studies were similar in type and distribution but of varying severity, consisting of scattered neuronal degeneration and necrosis, characterized by swelling and rounding of nerve cell bodies, increased eosinophilia, vacuolization of cytoplasm with a loss of Nissl substance (central chromatolysis), swelling and fading of nuclei, and separation and clumping of fibrillar and granular components of nucleoli. Only minimal changes were seen in the anterior sections, levels 1 and 2, even in high dosed animals. There was a striking distribution of lesions in the central nervous system; they were almost exclusively limited to levels 3, 4 and 5, corresponding to the pons and medulla. Cytopathic changes of injury were particularly marked in the paralemniscal nucleus, nucleus dorsalis raphae, nucleus pontis, nucleus vestibularis superior, the principal sensory nucleus (V), nucleus cochlearis dorsalis, nucleus cochlearis ventralis, and nucleus olivaris superior. Changes in the caudal medulla and thoracic spinal cord showed scattered axonal degeneration and necrosis characterized by swelling of axonal processes and spheroid formation. These lesions were most prominent in animals receiving 15 mg/kg/d or more. However, scattered neuropathic changes were also seen in the lowest dosed animals (5 mg kg d for 28 d), but in sections 4 and 5 only. No such lesion was seen in any reference control brain.

Rats. Histological evaluation of rat brains showed degenerating cell bodies in the central nervous system and spinal cord, morphologically and anatomically similar to those seen in dogs. Injury was predominantly at the level of the red nucleus and areas caudal to that structure in the brain-stem. Damage appeared to have a highly selective distribution within the hind brain with specific involvement of certain nuclei and cell groups. Involved nuclei in rats receiving artemether and arteether included the following. In the midbrain: red nucleus, dorsal cochlear, ventral cochlear, superior olive, trapezoid nucleus, ventral lateral lemniscus, dorsal lateral lemniscus, inferior colliculus, degenerated fibres to medial geniculate nucleus; in the hindbrain: reticular formation neuronal injury nucleus (reticular), pontis oralis nucleus (reticular), pontis caudalis nucleus (reticular), gigantocellularis nucleus, magnocellularis dorsal nucleus, medulla oblongatae centralis, ventral nucleus medulla oblongatae centralis; in the cerebellum: paramedian nucleus, lateral reticular nucleus, dorsal column nuclei: fasicular cuneatus and cuneatus externus vestibular nuclei, 'deep nuclei' (nucleus fastigii nucleus intermedius and lateralis) (PETRAS *et al.*, 1993).

Moderate to severe damage was consistently noted in the 25 and 50 mg/kg/d groups treated with both arteether and artemether. Silver staining confirmed prominent fibre degeneration in several areas, especially in the region of the superior olivary nucleus and fibre tracts to the medial geniculate nucleus (J.M.P.). Severity scores from sections at 5 levels showed significant differences in neuropathic scores by region with sections 3, 4 and 5 showing more evidence of injury at all dose levels (S.J.G.) ($P<0.01$, Kruskal Wallis). In the 3 section levels of the pons and medulla, damage appeared to be dose dependent (linear and logistic regression analysis; $P<0.01$) (Fig. 2).

Discussion

These studies showed a previously unrecognized severe toxicity of 2 artemisinin derivatives when administered daily in high doses to dogs or rats. The neuropathic lesion is reproducible, occurs in both species, and seems selectively to affect specific neuroanatomical areas in the caudal brain-stem area. There appeared to be little difference between the toxic effects of artemether and arteether.

The occurrence of the described lesions is of concern because of their nature, location and the lack of information regarding reversibility. Although other drugs of this class were not tested in these studies, artemether and other artemisinin analogues are now in clinical use, sometimes with multiple dose regimens over several days (HIEN & WHITE, 1993). Since these drugs are frequently used in severely ill or comatose patients in whom neurological sequelae are often seen, it might be difficult to recognize drug-induced neurotoxicity in a clinical setting.

The findings of drug associated neurotoxicity in 2 species should alert clinicians to possible neurotoxic effects of these drugs when used in high doses or for prolonged periods of time. Initial findings from our studies showed severe damage to the vestibular and auditory systems (PETRAS *et al.*, 1993). Therefore, early tests of hearing or vestibular function may be useful to detect brain stem injury at an early (and possibly reversible) stage in treated patients.

The mechanism of this neurotoxicity is unknown, but could be due to either prolonged exposure to parent drug or accumulation of toxic metabolite(s). A toxic metabolite may a more likely cause since neuropathic changes have not been reported even after much higher acute dosing, although seizures and cardiovascular changes have been noted (CHINA CO-OPERATIVE RESEARCH GROUP ON QINGHAOSU, 1982). This is also suggested by the poor correlation of parent drug levels to lesion severity (data not shown) and the delayed onset of symptoms in the high-dosed animals in our series (after 9 d in dogs and 5·5 d in rats). These drugs appear to be rapidly and extensively metabolized after absorption from intramuscular locations (J. O. Peggins, unpublished data; BENAKIS *et al.*, 1991), so accumulation of parent drug would be due to input from multiple i.m. sites. A major metabolite, dihydroartemisinin, appears to have a longer elimination half-life than the parent drug in both dogs and rats and accumulates during repetitive daily dosing (J. O. Peggins, unpublished data). Whether caused by the parent drug or metabolite, the highly specific anatomical nature of these lesions in our initial studies is consistent with targeted (i.e., receptor mediated) neurotoxicity).

Acknowledgements

This work was supported, in part, by WHO Tropical Disease Research grants nos. 870388 and 920548.

We gratefully acknowledge important assistance with statistical analysis from Mr Bob Burge and Dr Doug Tang, the superb technical assistance of M. Sperry, L. Figueroa, A. Aguilar, M. Vane and C. King and the excellent secretarial assistance of M. Bell.

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